Serum anti-mullerian hormone level and antral follicle count in patients with endometriomas pre and post endometriotic surgery for assessment of ovarian reserve

Thesis

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قالوا سَنَحَاقِكِ لَا عَلِمَ لَنا إِلَّا مَا عَلَمْتَ إِنَّكَ أَفْتَتَ الْعَلِيمَ الْحَكِيمَ

صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ
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Abstract

Endometriosis is an important disease that affects monthly fecundity rate or the success of the assisted reproductive technologies (ART). The prevalence of endometriosis is approximately 6–8%, and it is usually diagnosed during laparoscopic surgery for the evaluation of pelvic pain. The prevalence of endometriosis is higher among infertile women than fertile. Of the surgical population, endometriosis was diagnosed in 25% of women who had a laparoscopy for pelvic pain and in 20% of women who underwent surgery for infertility.

Endometrioma is one of the most commonly encountered diagnoses in ovarian surgery and may be present in up to 17-44% of patients with endometriosis. Ovarian endometriomas are usually associated with the symptoms of dysmenorrhea, chronic pelvis pain, dyspareunia, and infertility. Previous studies have demonstrated that endometriomas can negatively affect the rate of spontaneous ovulation, as well as reducing the amount of follicular number and activity in the adjacent ovarian tissues.

Therapeutic approach for women with ovarian endometrioma may vary according to the age of women, size of the cyst, symptoms, and desire for future conception. Laparoscopic cystectomy for endometrioma is common and seems to be feasible in terms of postoperative fecundability and recurrence rate compared with that of fenestration and coagulation of the cyst wall. However, the safety of this technique with respect to residual ovarian damage has been questioned.

Key words

Endometriosis
Ovarian endometriomas
Open and laparoscopic surgery of ovarian endometriomas
Ovarian reserve
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<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>IVF</td>
<td>In-vetro fertilization</td>
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<tr>
<td>ICSI</td>
<td>Intra-cytoplasmic sperm injection</td>
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<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>CC</td>
<td>Clomiphene citrate</td>
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<tr>
<td>GnRH</td>
<td>Gonadotrophin-releasing hormone</td>
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<tr>
<td>AMH</td>
<td>Anti-Mullerian hormone</td>
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<tr>
<td>AFC</td>
<td>Antral folliclecount</td>
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<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
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<tr>
<td>E2</td>
<td>Estrione</td>
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<tr>
<td>ABC</td>
<td>Argon Beam coagulator</td>
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<tr>
<td>APC</td>
<td>Argon plasma coagulator</td>
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<tr>
<td>ART</td>
<td>Assisted reproductive technologies</td>
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<tr>
<td>AMHRII</td>
<td>Anti-Mullerian hormone receptor type II</td>
</tr>
<tr>
<td>BMPs</td>
<td>Bone morphogenetic proteins</td>
</tr>
<tr>
<td>PCOS</td>
<td>Polycystic ovary syndrome</td>
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<td>OHSS</td>
<td>Ovarian hyper stimulation syndrome</td>
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<tr>
<td>HCG</td>
<td>Human chorionic gonadotropin</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunesorbent assay</td>
</tr>
<tr>
<td>HE4</td>
<td>Human epididymal secretory protein E4</td>
</tr>
<tr>
<td>ASRM</td>
<td>American Society for Reproductive Medicine</td>
</tr>
<tr>
<td>EOC</td>
<td>Epithelial ovarian cancer</td>
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<tr>
<td>EAOC</td>
<td>Endometriosis-associated ovarian carcinoma</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>TAFC</td>
<td>Total antral follicle count</td>
</tr>
<tr>
<td>R</td>
<td>Correlation coefficient</td>
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<tr>
<td>IVF-ET</td>
<td>In-vitro fertilization and embryo transfer</td>
</tr>
<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
</tr>
<tr>
<td>HRP</td>
<td>Horseradish peroxidase</td>
</tr>
<tr>
<td>TMB</td>
<td>Tetra-methyl benzidine</td>
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</table>
Endometriosis affects approximately 10% of the female population in their fertile years (Eskenazi et al., 2001).

Ovarian endometriomas are a common form of the disease and may be present in up to 30–40% of women with endometriosis (Vercellini et al., 2006).

Ovarian reserve is a term used to describe the functional potential of the ovary and reflects the number and quality of oocytes. An ideal test of ovarian reserve should predict both ability and inability to have a live-born baby with or without treatment. In an ideal world, it should also predict preservation of current levels of ovarian activity. A growing body of evidence suggests that ovarian reserve is damaged after excision of ovarian endometriomas (Tsoumpou et al., 2008; Garcia-Velasco and Somigliana, 2009).

The ovulation rate has been repeatedly shown to be reduced in operated gonads compared with contralateral intact gonads. Moreover, data from IVF–ICSI cycles consistently showed a decreased ovarian responsiveness to hyper-stimulation in previously operated ovaries. The damage inflicted by surgery to ovarian reserve may be due to the removal of healthy tissue by laparoscopic stripping, the surgery-related local inflammation or vascular compromise following electrosurgical coagulation (Tsoumpou et al., 2008).

There are two types of tests of ovarian reserve: static and dynamic. Static tests assess specific parameters relating to ovarian reserve at a single point in time and involve both ultrasound and biochemical parameters. Dynamic tests assess ovarian response to exogenous stimulation. Usually this involves measurement of hormonal concentrations in a serum sample before and after stimulating the ovaries
using FSH, clomifene citrate (CC) or a gonadotrophin-releasing hormone (GnRH) agonist (Broekmans et al., 2006).

Anti-Mullerian hormone (AMH) has been recently acknowledged as the most useful, reliable, and sensitive hormonal serum marker of the ovarian primordial follicle pool compared with other known serum markers (Van Rooij et al., 2002).

Furthermore, serum AMH levels are strongly correlated to antral follicle count (AFC) measured by ultrasound (De Vet et al., 2002).

This relationship was documented more reliable by AMH than those obtained with serum levels of inhibin B, E2, FSH, and LH (Fanchin et al., 2003).

In addition, the AMH level represents a stronger independent marker of ovarian reserve without significant fluctuation during the menstrual cycle, which progressively decreases with age (Tsepelidis et al., 2007; Van Rooij et al., 2005).
This is a prospective study comparing ovarian reserve following surgical treatment of endometriomas by either traditional cystectomy or ablation of endometriomas using Argon Beam coagulator (ABC).
Anti-Mullerian hormone (AMH) is a dimeric glycoprotein, a member of the transforming growth factor-beta super-family which acts on tissue growth and differentiation. AMH was originally identified because of its fundamental role in male sex differentiation. Indeed, expressed in the Sertoli cells of fetal testis, AMH induces the regression of the Mullerian ducts. In the absence of AMH, Mullerian ducts evolved into uterus, fallopian tubes and the upper part of the vagina. In women AMH is produced by granulosa cells, from pre-antral and antral follicles and the main physiological role of AMH in the ovary seems to be limited to the inhibition of the early stages of follicular development (*La Marca et al*, 2010).

AMH is secreted by the ovary into circulation; hence AMH is measurable in serum. As serum AMH levels essentially reflect the ovarian follicular pool, reduction in the number of small growing follicles may be followed by a reduction in circulating AMH. In particular in the last few years several large prospective studies have been published reporting extremely interesting new data on the possible clinical application of AMH measurement in the prediction of quantitative and qualitative ovarian response in assisted reproductive technologies (ART) (*La Marca et al*, 2010).

**AMH in ovarian physiology**

AMH is produced by granulosa cells from pre-antral and antral follicles, restricting expression to growing follicles, until they have reached the size and differentiation state at which they are selected for dominance by the action of pituitary FSH (*Weenen et al*, 2004).

In the human this occurs in antral follicles of size 4–6 mm, AMH is not expressed in atretic follicles and theca cells. Ovarian AMH expression
Anti-Mullerian hormone has been observed as early as 36 weeks’ gestation in the humans’ fetus. Recent studies show that in adult rat ovaries FSH and estradiol may down-regulate AMH expression. AMH exerts its biological effects through a trans-membrane serine/threonine kinase type II receptor (AMHRII), which is specifically expressed in the gonads and in the mesenchymal cells adjacent to the Mullerian ducts (Broekmans et al, 2008).

In adult female rats, AMH and AMHRII mRNAs are mainly expressed in granulosa cells from pre-antral and smaller antral follicles. In addition, AMHRII mRNA expression was observed in theca cells of pre-antral and small antral follicles. Besides the exclusive AMHRII, three candidate AMH type I receptors have been identified to be involved in AMH-induced Mullerian duct regression. These type I receptors are shared with the bone morphogenetic proteins (BMPs). Subsequently, similar to BMPs, AMH signaling is mediated through the downstream signaling molecules Smad1, Smad5 and Smad8. However, the relative contribution of these three type I receptors to AMH signaling in the ovary remains to be determined. The main physiological role of AMH in the ovary seems to be limited to the inhibition of the early stages of follicular development, since both in vivo and in vitro experiments have indicated that the transition from primordial into growing follicles becomes enhanced in absence of AMH, leading to early exhaustion of the primordial follicle pool. In vitro culture of mouse neonatal ovaries and human cortical strips has confirmed the inhibitory role of AMH in primordial follicle recruitment (Carlsson et al, 2006).

Moreover it has been suggested that follicles are more sensitive to FSH in the absence of AMH. The effects of AMH on FSH sensitivity of follicles was tested in an in vivo model in which the follicle dynamics
were compared with wild-type and AMH null mice in the presence of low and high FSH serum concentrations. The study shows that more growing follicles were found in AMH null mice than in wild-type mice, both in terms of numbers and in terms of developmental stage (*Broekmans et al., 2008*).

**Figure (A).** AMH is secreted by pre-antral and antral follicles.

It seems to inhibit initial follicle recruitment and FSH-stimulated follicle growth. The role of AMH in the two main compartments of normal ovarian follicle development (the red centre represents the oocyte, the grey area represents the granulosa cell layer and the white area represents follicle fluid in the antrum). AMH is expressed in small and large pre-antral follicles (broken arrows) and in small antral follicles (whole arrow), and the latter mainly contributes to serum levels. Initial recruitment takes place as a continuous process, whereas cyclic recruitment is driven by a rise in FSH serum levels at the end of a previous menstrual cycle. The inhibitory effects of AMH are shown (a) on the initial recruitment of primary follicles from the resting primordial follicle pool and (b) on the sensitivity of antral follicles for FSH (reproduced with permission from *Broekmans et al., 2008*).

Recently, ovaries from rats placed in organ culture and incubated in the absence and presence of AMH; show that AMH alters the expression of several hundred genes. The overall effects of AMH exposure was to decrease the expression of stimulatory factors, increase the expression of inhibitory factors and regulate cellular pathways that result in the inhibition of primordial follicle development (*Nilsson et al., 2007*).
Current theories also suggest a role for AMH as a co-regulator of steroidogenesis in granulosa cells, as AMH levels appear to be related to estradiol levels in follicular fluid from small antral follicles. This is confirmed by a recent study which showed that polymorphisms in the gene for AMH or AMH receptor type II seem to be related to follicular phase estradiol levels, suggesting a role for AMH in the FSH-induced steroidogenesis in the human ovary (Kevenaar et al., 2007).

Factors modulating AMH levels in women

AMH is produced and secreted by the gonads into the circulation, and AMH is measurable in serum from both men and women. Serum AMH levels from women are lower than those in men throughout life. In women AMH levels are almost undetectable at birth with a subtle increase within the first 2 or 4 years of age, after that AMH appears to be stable until adulthood but found to decrease as a sign of follicular reserve exhaustion becoming undetectable at menopause (La Marca 2009a).

Interestingly, in women circulating AMH appears to be solely of ovarian origin since AMH is undetectable 3–5 days following bilateral ovariectomy. As AMH levels essentially reflect the follicular ovarian pool, reduction in the number of small growing follicles may be followed by a reduction in circulating AMH. The reduction in ovarian reserve is a physiological process occurring in the late reproductive period and consistently associated with a decrease in AMH levels (Robertson et al., 2008).

The strong correlation existing between AMH levels and the resting pool of follicles has recently been highlighted by some papers showing that AMH measurement may be used to predict the occurrence of menopause (Sowers et al., 2008; Van Disseldorp et al., 2008).
Non-significant variations of AMH throughout the menstrual cycle have been reported by our group and confirmed by a number of independent studies others have reported significant cyclical fluctuations in AMH levels with a rapid decrease in the early luteal phase. However, excursions from mean levels of 3% to 219% have been calculated. These variations are similar to reported inter-cycle variability for AMH. Hence in the clinical setting the inter- and intra-cycle variability in serum AMH levels may be considered to be low enough to permit random timing of AMH measurement during the menstrual cycle (La Marca et al, 2010).

In women, AMH levels seem to be unmodified under conditions in which endogenous gonadotrophin release is substantially diminished, such as during pregnancy, GnRH agonist treatment and short-term oral contraceptive administration, indicating that non-cyclic FSH-independent

Figure (B) Left: Mean serum AMH levels show a reduction throughout reproductive life. Undetectable AMH levels after spontaneous menopause have been reported (constructed graphic). Right: Circulatory pattern of AMH during the menstrual cycle of young healthy women aged 18–24 years. Serum AMH levels have been shown to be stable throughout the menstrual cycle. Day 0 ¼ day of LH surge (reproduced with permission from (La Marca et al., 2010).
Ovarian activity persists even when pituitary FSH secretion is suppressed (La Marca et al, 2010).

Women with polycystic ovary syndrome (PCOS) show increased development of antral follicles compared with normal women. On histological examination, polycystic ovaries (PCO) exhibit a normal number of primordial follicles, whereas the number of developing follicles is double compared with normal ovaries. Accordingly circulating AMH levels in women with PCOS are two to three times higher than healthy controls (Wachs et al., 2007).

In women with PCOS, increased AMH levels may not only be due to excessive accumulation of antral follicles but also to increased granulosa cell AMH secretion (Wang et al., 2007).

Indeed, levels of AMH are on average 75 times higher in granulose cells from PCO, compared with levels in granulosa cells from normal ovaries (Pellatt et al., 2007).

AMH levels appear to be related to the severity of the syndrome, since levels have been observed to be higher in insulin-resistant PCOS women than in patients with normal insulin sensitivity. Similarly AMH is higher in amenorrheic compared with oligomenorrheic women with PCOS, which could indicate a role for AMH in the pathogenesis of PCOS-related anovulation. The relationship between AMH levels and the severity of the syndrome seems to be confirmed by studies demonstrating that PCOS patients ovulating during a weight loss-program had AMH levels lower than women remaining anovulatory. Interestingly, in one study no significant changes in AMH levels were observed in either responders or non-responders during the weight loss-program (Thomson et al., 2009).
In order to clarify the complex relationship existing between insulin resistance, androgen excess and high levels of AMH, a prospective, randomized, doubleblind 26 week-long study was undertaken in women with PCOS. All patients received diet and lifestyle counseling, and metformin. Concomitantly, they were randomized to either dexamethasone or placebo. The study clearly demonstrated that circulating AMH concentrations were unaffected by 6 months of lifestyle counseling with metformin and placebo treatment. AMH levels were also unaffected by 6 months of androgen suppression with dexamethasone in addition. These results may indicate that high serum AMH levels in PCOS may be more strongly related to the presence of PCO than to the full spectrum of the syndrome (PCOS) as modifications in androgens and insulin sensitivity are not followed by changes in ovarian AMH output (Carlsen et al., 2009).

Finally, AMH measurement has been found to offer a relatively high specificity and sensitivity (92 and 67%, respectively) as a diagnostic marker for PCO. On this basis it has been proposed that in situations where accurate ultrasound data are not available, AMH could be used instead of the follicle count as a diagnostic criterion for PCOS (Pigny et al., 2006).

Obesity has been associated with reduced fertility, even in the presence of ovulatory menstrual cycles, and to increased probability of miscarriage compared with normal weight women. Non-PCOS obese women show reduced levels of inhibin B and AMH suggesting that obesity may be associated with impaired ovarian reserve (Freeman et al., 2007).

However, a recent study examined the correlation of obesity with hormonal and ultrasound derived markers of ovarian reserve and found